10. A method for obtaining an antibody having specific binding affinity for human erythropoietin receptor polypeptide, said method comprising:

contacting a non-human mammal with a purified preparation of an extracellular domain fragment of human erythropoietin receptor polypeptide, wherein the fragment contains only native human erythropoietin receptor, and

collecting said antibody from said non-human mammal.

REMARKS

This Preliminary Amendment is submitted for entry in the above-identified application prior to an Examiner undertaking a first Action in connection therewith. The Examiner's new matter rejection indicated at ¶3 of Paper 19 (09/26/00) has been alleviated by removal of the indicated subject matter included in the preliminary amendment of January 11, 1999. The amended claims are fully supported by the specification.

35 U.S.C. § 112

In response to Examiner's rejections in ¶6a and ¶6b of Paper 25 (7/13/01 Office Action) the term "unglycosylated" and reference to sequence identifiers have been removed. The claims now are in compliance with 35 U.S.C. § 112.

35 U.S.C. § 102(b)

Claims 3-5 and 8 were rejected as being anticipated by Harris *et. al.* (*JBC*, 1992), subsequently referred to as Harris. Harris teaches the production of a fusion protein containing the extracellular domain of erythropoietin (Epo) receptor. However, Harris loses most of the protein product into an non-functional, insoluble pellet, thereby not successfully producing high levels of the active polypeptide. Harris, Page 15206, col. 1, lines 39-43. The isolation and purified extracellular domain of the Epo receptor from the fusion protein and other cellular components is not taught by Harris. The antibodies of the present invention are directed against only the extracellular domain of the Epo receptor, because only the purified extracellular domain is introduced to the antibody producing mammal. Harris produces antibodies to the GST fusion protein, and therefore cannot assert that the antibodies produced recognize only the extracellular domain of

Epo receptor with specificity. Harris also does not teach the use of the antibodies produced in solid-phase assays as taught in the present invention.

The examiner asserts that because Harris teaches that the antibodies block the binding of the radiolabeled Epo to the cellular receptor, this meets the functional limitations of claims 4 and 8. Claims 4 and 8 require specific binding of the antibody to the extracellular domain of Epo receptor. Harris does not require, assert or show any antibodies that meet this limitation. Harris raises and purifies antibodies using the fusion protein. Therefore antibodies recognizing the GST domain either alone or in combination with the Epo receptor are not eliminated. Harris's challenge experiment supposedly showing prevention of binding the Epo to the receptor expressed on a cell is flawed. Harris uses a huge quantity of his antibody, 500 µg/mL and allows binding for 30 minutes. At this concentration level, the antibody will bind everywhere, coating the cell surface and the receptor. Specificity of the antibody for the extracellular domain of the Epo receptor is not demanded or successfully challenged with only one washing step. Then Harris introduces an amount of Epo, 0.1 nm, which is 10 x more dilute that the binding constants he measured *in vitro* for Epo to its receptor. At this low concentration it is not surprising that the radiolabeled Epo could not challenge the bound antibodies for access to the receptors. Harris does not show specificity of the antibodies for the extracellular domain as demanded by the present invention.

35 U.S.C. § 103

Examiner rejected claims 3-6 and 8-10 as being unpatentable over Harris in view of D'Andrea '808. Applicant agrees with the Examiner that Harris does not show the use of a polypeptide consisting of only the extracellular domain of Epo receptor as an immunogen, nor shows the use of antibodies in solid-phase assays. D'Andrea does not combine with Harris to render the present invention obvious. D'Andrea utilizes antibodies to Epo. D'Andrea does not teach the production of antibodies to the Epo receptor or the extracellular domain of the Epo receptor. D'Andrea does not teach the purification of antibodies to the Epo receptor or the extracellular domain of the Epo receptor. Furthermore, D'Andrea does not teach the use of antibodies to solid-phase immunological reagents as taught in the present invention. D'Andrea does not utilize nor suggest the use of fusion proteins for protein purification of the present invention. Neither Harris nor D'Andrea

show successful high level production of an Epo receptor extracellular domain fusion, where the extracellular domain can be cleaved from the fusion and protein and highly purified.

Applicant respectfully submits that the application is now in condition for allowance.

The Commissioner is authorized to charge any additional fees associated with this paper or credit any overpayment to Deposit Account No. 11-0982.

Respectfully submitted,

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Date: Sep. 10, 2002 By: _____

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APPENDIX:
MARKED UP VERSION OF SPECIFICATION AND CLAIM AMENDMENTS

3. A fusion protein consisting of an upstream portion, a cleavage site, and a [purified] human erythropoietin receptor polypeptide consisting of a human erythropoietin receptor extracellular domain [about amino acid 25 to about amino acid 250 of the full length human erythropoietin receptor protein of SEQ ID NO:5], said human erythropoietin receptor polypeptide being capable of binding human erythropoietin, wherein said [polypeptide is non-glycosylated] fusion protein expressed at high levels, purified by affinity chromotography and cut at the cleavage site producing free human erythropoietin receptor extracellular domain.

4. A purified antibody having specific binding affinity for [a purified] human erythropoietin receptor [polypeptide], [said polypeptide consisting essentially of about amino acid 25 to about amino acid 250 of the full length human erythropoietin receptor protein, said polypeptide being capable of binding human erythropoietin] said antibody produced against a purified fragment of human erythorpoietin receptor extracellular domain, wherein the fragment contains only amino acids corresponding to the human erythropoietin receptor.

5. An immunoassay compostion comprising:
(a) a solid phase immunoassay reagent; and
(b) the protein of claim 3 operably coupled to said reagent.

6. An immunoassay compostion comprising:
(a) a solid phase immunoassay reagent; and
(b) an antibody of claim 4 operably coupled to said reagent.

8. A purified antibody having specific binding affinity for a purified human erythropoietin receptor extracellular domain polypeptide, [said polypeptide consisting essentially of about amino acid 25 to about amino acid 250 of the full length human erythropoietin receptor protein,] wherein said polypeptide is [unglycosylated] expressed in E-coli, capable of binding human erythropoietin and does not include any amino acids from non-human DNA.

9. An immunoassay compostion comprising:
(a) a solid phase immunoassay reagent; and
(b) an antibody of claim 8 operably coupled to said reagent.

10. A method for obtaining an antibody having specific binding affinity for [an purified] human erythropoietin receptor polypeptide, [said polypeptide consisting essentially of about amino acid 25 to about amino acid 250 of the full length human erythropoietin receptor protein (SEQ ID NO:5), wherein said polypeptide is unglycosylated], said method comprising:
contacting a non-human mammal with a purified preparation of an extracellular domain fragment of human erythorpoietin receptor polypeptide, wherein the fragment contains only amino acids corresponding to the human erythorpoietin receptor polypeptide, and
collecting said antibody from said non-human mammal.